SPERMATOGENESIS OF THE PIG WITH SPECIAL REFERENCE TO THE ACCESSORY CHROMOSOMES.

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INTRODUCTION.

In late years the occurrence of accessory chromosomes, in various degrees of complexity, has been recorded from diverse groups of invertebrates. Recently the field has been extended to the vertebrates, Guyer having shown their existence in the males of the guinea ('09*a*), rooster ('09*b*), rat ('10), and man ('10); Newman and Patterson ('10) in the armadillo; Jordan ('11) in the opossum; Stevens ('11) in the guinea-pig; and King ('12) in *Necturus*. The existence of what appears to be such an element has also been reported in the ovary of the cat by Winiwarter and Saintmont ('09). In a recent paper Jordan ('13) states that the heterochromosomes are unquestionably lacking in the pig. I find, however, that the accessory chromosomes are very conspicuous in all of the pig material which I have studied.

Wilson ('09) found in *Syromastes* that half of the spermatids possess two more chromosomes than the remainder. It was predicted by him that in consequence the somatic cells of the female of this species would show two more chromosomes than the somatic cells of the male, and later the facts were found to be in exact accord with his predictions, the somatic cells of the female of *Syromastes* having been found to contain twenty-four, those of the male, twenty-two chromosomes. A similar condition has been found in other tracheates, but dimorphism in the number of chromosomes in the germinal and somatic cells of the two sexes among the vertebrates has thus far been only inferred, not actually demonstrated.

The present study on the spermatogenesis of the pig was taken up at the suggestion of Professor M. F. Guyer, to whom I am much indebted for many helpful suggestions during the progress of the work. Points of special interest in this paper are as follows:

1. The presence of a distinct pair of accessory chromosomes.
2. The resulting dimorphic condition in the spermatozoa of the pig.
3. Dimorphism in the number of chromosomes in both the germinal and somatic cells of the male and female animals.
4. The abundance of large conspicuous interstitial cells in the testes.
5. The second reduction of the chromosome number in the secondary spermatocyte, which was found to be simply equational.
6. The throwing off of a large mass of cytoplasm, containing one of the centrosomes, at the time of the final development of the spermatozoan.

Material and Methods.

I have been very fortunate in obtaining from several sources exceptionally good material for this investigation. The major part of the material studied was obtained from a vigorous Poland China boar about ten months old. The animal came from registered stock and was the property of the College of Agriculture of the University of Wisconsin. The material was ob-

tained through the courtesy of Professor L. J. Cole. Immediately after the testes were removed from the live animal, pieces were placed in various kinds of fixing fluids, as Bouin's, Zenker's, Tellyesnicky's, and corrosive-acetic.

Sections from various parts of the testes were made from four to twelve microns thick and while several methods of staining were employed, material fixed in Bouin's fluid and stained with Heidenhain's iron hematoxylin with acid fuchsin as a counterstain, proved to be the most satisfactory. Delafield's hematoxylin with eosin counterstain was also used and gave favorable results. While material fixed in some of the other fluids mentioned was fairly good, the first method proved to be so superior that the sections used in this study were almost wholly those prepared according to it. Other material, from a much older animal, was studied in sufficient detail to corroborate the results obtained through the study of the material secured from the younger and more vigorous animal.

I also owe many thanks to Professor B. M. Allen for his generosity in placing at my disposal his embryological material of pigs of both sexes. The material in question, which was that used by Dr. Allen ('04) in his own researches on the embryology and development of the ovary and testes in mammals, was fixed in Flemming's fluid and stained with Heidenhain's iron hematoxylin mainly. By examining a large number of these slides, I not only corroborated my previous count of the spermatogonial number of chromosomes, but was also able to make fairly conclusive counts of the chromosomes in the somatic cells of the male, and in the oögonia and somatic cells of the female. Without the use of his material this important phase of the problem could not have been worked out at this time.

General Arrangement of the Germinal Cells.

The structure of the testes of the pig does not differ greatly from that of other well-known mammals, except for the presence of numerous masses of large well-defined interstitial cells which are scattered among the seminiferous tubules and comprise about one fourth of the entire volume of the testes (Fig. 1). A detailed description of these interesting cells, which contain

numerous mitochrondia, will be reserved for a separate paper. The germinal cells themselves are found in a great number of seminiferous tubules coiled throughout the interior of the testes. Sections of the tubules are found in groups of fifteen to twenty-five which are completely surrounded by walls of connective tissue. This connective tissue forms a sort of continuous network of walls throughout the testes and is rather thick in places, usually where three or four of the groups become appressed. Embedded in these walls are blood- and lymph-vessels which branch out into the masses of interstitial cells between the tubules.

The arrangement of the cells in the tubules is similar to that of the other warm-blooded animals and the usual four types of germinal cells are present. The spermatogonia form a more or less regular layer of cells lying next to the wall of the tubule. These divide and give rise to two new cells, one or both of which may become the primary spermatocyte. The primary spermatocytes are very abundant, especially in the spireme stage. After various changes and considerable growth the primary spermatocytes divide and give rise to the secondary spermatocytes. The secondary spermatocytes divide in turn to form the spermatids, which transform directly into spermatozoa (Figs. 64–78).

The material studied must have been in maximum activity at the time of fixation, for the four types of germinal cells were easily found in stages of growth and development. Mitotic stages of spermatogonia, primary and secondary spermatocytes, were very abundant, and frequently the entire field under the oil immersion lens was composed of cells in mitosis.

Spermatozoa in all stages of development were very abundant, and clusters of the cells which were in the final stages of development could be seen attached to the long cylindrical Sertoli cells which often extend to the lumen of the tubule (Figs. 2 and 3). The Sertoli cells, which are quite abundant, are closely connected with the germinal cells and will be described in more detail later (Fig. 63).

Spermatogonia.

The spermatogonia usually lie in a single layer next to the wall of the tubule though occasionally some of the cells are

crowded out, thus forming a second layer which is always very irregular. There is considerable variation in the appearance and some variation in the size of different spermatogonia. Very frequently the cells are far apart, in which case they are flattened out on the tubule wall.

The amount of cytoplasm is small and in some of the earlier stages the cell boundaries are very indistinct (Fig. 16). Later the nuclei assume a round shape and the cell wall becomes visible. The amount of chromatin material increases and the nuclei at this stage resemble very much the nuclei of the large interstitial cells both in appearance and size (Fig. 17).

Two large nucleoli much alike in size seem to be present in all stages of these cells. Even in the testes of young embryos the two bodies are very conspicuous. Besides these large nucleoli a varied number of smaller, similarly staining bodies are usually present, two of which seem to occur most frequently, and evidently make their appearance first (Figs. 16 and 17). Later other small nucleoli appear. At present I am unable to attach any meaning to them as their numbers are so varied in the various cells. The two large nucleoli, however, are very constant, and I have been able to trace them throughout the entire spermatogenesis. From all appearances one is led to believe that these nucleoli and the accessory chromosomes are one and the same, a condition similar to that found by Guyer ('10) in man, and by others in some of the lower forms. Jordan ('11), however, has not been able to identify any such structures at this stage as the future accessory chromosomes in the opossum.

At the conclusion of the resting stage numerous chromatin granules appear, which arrange themselves along fine threads in an entangled mass. The two nucleoli come in close contact and the nuclear membrane gradually disintegrates. Eighteen chromosomes appear in the late prophase of the spermatogonial division (Fig. 18). Sixteen of these are rod-shaped, variously curved and somewhat different in size. Two, which are always found close together, and frequently off to one side are slightly larger and oval in shape. The sixteen ordinary chromosomse or autosomes arrange themselves with the two accessory chromosomes in the equatorial plate (Fig. 19). The accessories, like

the autosomes, divide in this stage. A centrosphere containing a tiny centrosome can be observed in some cases, but its presence is not at discernible in these early cells as it is in the later stages.

Primary Spermatocytes.

1. *Resting Stage.*

The primary spermatocytes which arise from the final spermatogonial division in the early resting stage are usually somewhat smaller than the spermatogonia in the metaphase and prophase stages. The two large nucleoli are quite conspicuous and remain black in sections stained with iron hematoxylin even in very much destained material (Fig. 20). Usually two small nucleoli, stained the same way as the large ones, are plainly visible and occasionally only one, or more than two of these bodies appear.

2. *Synizesis and Growth Period.*

An increase in the bulk of both the nucleus and cytoplasm begins after a brief period of rest. The chromatin appears to be arranged in much the same way as it was during the resting condition of the spermatogonia, except that the linin fibers are coarser. The cytoplasm is composed of granular masses and clear, apparently liquid areas. The chromatin threads and nucleoli become massed at one side of the nucleus (Fig. 21). The nuclear wall expands and the clear area formed by the massing of the chromatin to one side becomes much enlarged. The clear areas in the cytoplasm decrease coincidentally with the increase of the liquid-like area in the nucleus and one is led to believe that the cytoplasmic fluid permeates the nuclear wall during the period of synizesis.

During the collapse of the chromatin material, the nucleoli can be plainly seen especially in well destained sections (Fig. 21). They are usually in that portion of the chromatin mass which is nearest to the nuclear wall. Opposite this mass on the outside of the nuclear wall a centrosphere can often be seen, but the centrosome is rarely visible at this stage. The chromatin threads become arranged in a very much tangled mass of loops which later appear in about half the original number and fully twice as thick. (I have not been able to determine definitely whether

the pairing takes place by telo- or parasynapsis.) The whole mass then moves toward the center (Fig. 22), the large clear area in the nucleus disappears, and the nuclear wall becomes spherical and clearly defined (Fig. 23). While the formation of the spireme is going on, the cytoplasm, too, increases greatly in volume and appears very granular. The cell now is fully twice as large as a spermatogonium and judging by the large number of cells that can be seen in the spireme stage one can safely conclude that they remain in that condition for some time. The spireme finally breaks up into U and variously shaped chromosomes (Fig. 25). The two large nucleoli, which remain in full view throughout this stage, become oblong and can often be seen close together (Fig. 25).

3. *Reduction Division.*

The primary spermatocytes when ready for division reveal ten chromosomes in the late prophase or early metaphase stage. The two accessories which are ordinarily off to one side (Figs. 8, 26, 27, and 29) can be recognized at a glance. The other chromosomes are usually arranged in a ring. Judging from their large size and changed form, they are bivalent, representing the paired univalent chromosomes of the spermatogonium. That is, of the original eighteen chromosomes, sixteen have paired to form eight bivalents of the primary spermatocyte and two have remained unpaired as the accessory chromosomes. Sometimes, although ten chromosomes can be counted, it is difficult to tell just which are the accessories owing to overlapping. The chromosomes differ somewhat in size as can be seen in Figs. 26 to 29. Figs. 9 to 12 and 30 to 37 show the accessories in characteristic positions in the metaphases of division of the primary spermatocyte. They always pass entire, side by side and in advance of the divided autosomes, toward one pole. This is possibly due to the fact that they are not retarded by division.

The fact that the chromosomes immediately after divergence (Fig. 28) resume the appearance (except in size) that characterizes the univalent spermatogonial chromosomes, and also because the accessory chromosomes pass over entire to one pole in this division while they are halved in the next division, seems to indicate strongly that this is the reduction division.

It is obvious that as far as chromatin content is concerned the division of the primary spermatocyte gives rise to two dissimilar cells, one of which receives eight chromosomes, and the other eight plus the two accessories or ten chromosomes (Fig. 38). Figure 39 is a drawing of one end of a late anaphase of such a division showing eight chromosomes, and Fig. 40 shows the other end which received eight of the ordinary chromosomes and the double accessory.

Occasionally a small chromatin body is present in this first spermatocytic division (Figs. 28, 31, 32, 35 and 37). Figure 31 shows such a body passing to the same pole with the accessories, in advance of the other chromosomes. Figure 32 represents an earlier stage of much the same thing. In Fig. 35 it can be seen passing to the opposite pole, and Fig. 37 represents an extremely rare case where two such bodies are present, one somewhat larger, passing to either pole, even in advance of the two accessory chromosomes. Figure 28 shows the body outside of the main ring of chromosomes. While the small body can be seen frequently, as a rule no such an element can be detected, and while it may possibly be comparable to the small pair of chromosomes found so constantly in some of the Tracheata, my present data on its irregular occurrence and behavior do not permit a conclusion regarding its significance.

Secondary Spermatocyte.

1. *Dimorphism.*

The dimorphism of the secondary spermatocytes, which resulted from the last division, is again expressed in the resting stage that sometimes follows. Approximately half of them showed, under proper decolorization, two large chromatin nucleoli (Fig. 41) while in the others only the small nucleoli appeared (Fig. 42). The nucleoli retain the usual deep staining capacity, and as was true in the previous stages, even when all the other material is almost totally decolorized, the nucleoli remain very conspicuous. Frequently, both primary and secondary spermatocytes were found dividing in the same field, which fact seems to suggest that at times there is no intervening period of rest between the two divisions, or that it is very brief. Figure 43

shows the two resulting cells of a primary spermatocyte division which are still in close contact and both ready for dividing into spermatids. Resting stages, however, appear in abundance and in this stage, as was the case in the resting stage of the primary spermatocyte, the centrosome surrounded by a clear zone becomes large and very conspicuous (Figs. 41 and 42).

2. *Second Reduction Division (Equational).*

Although the division of the primary spermatocyte gave rise to cells containing eight and ten chromosomes respectively (Figs 38, 39, 40), when these cells become ready for division half of them show four (Figs. 13, 14, 43, 45, 46) and the other half six chromosomes (Figs. 43, 44, 47). Thus a second pairing of the ordinary chromosomes, similar to that found by Guyer in the pigeon ('00), corroborated by Geoffrey Smith ('12), man ('10), guinea ('09*a*), and chicken ('09*b*), and by Jordan in opossum ('11), has evidently taken place so that there are four bivalents in each type of cell and the additional two accessory chromosomes in the one type. Stevens ('11) says that there is no such second synapsis or numerical reduction in the guinea-pig. Figures 45 and 46 show four large chromosomes in the metaphase stage of one type of secondary spermatocyte and Figs. 44 and 47 represent the other type in which six chromosomes appear, four bivalent autosomes plus the two accessories. The four chromosome group is evidently formed by the pairing of the eight chromosomes of one type of cell resulting from the first maturation division at one pole, and six chromosome group is interpreted as being derived by the pairing of the eight chromosomes plus the two unpaired accessories at the opposite pole (Figs. 38, 39, 40).

Guyer ('10) in speaking of the second conjugation of the chromosomes in man says:

"Assuming that the respective chromosomes are more or less qualitatively differentiated, such a numerical reduction, however, by no means necessarily implies that there has also been a second qualitative reduction. Aside from the improbability of such a reduction, the general appearance of the divided chromosomes would not warrant this interpretation; for instead of the elongated univalent type as seen in the spermatogonia, or in ana-

phases of the divisions of spermatocytes of the first order, the daughter chromosomes here retain the rounded appearance and increased size that is characteristic of the bivalent types (compare Figs. 1, 10, and 11, 13, 14, 15, 16 and 17). Thus while half of the spermatids receive five, and half seven chromosomes, in terms of univalence the numbers would in all probability be ten and twelve respectively."

Jordan ('11) in speaking of the same condition in the opossum remarks as follows:

"Similarity of form between the chromosomes of the first and second metaphase plates (*i. e.*, double rods) suggests a similar manner of division: accordingly a second reduction. When one recalls, however, that a resting stage (Figs. 39 to 41) usually intervenes between the first and second maturation divisions, when the chromosomes pass through a reticular phase, the above conclusion is inadmissible; or rather, no definite conclusion respecting the character of the second division is justified. A double true reduction, suggested by the form of the chromosomes is contrary to our fundamental conceptions regarding the significance of chromosomes and need not, in view of the nature of the evidence, be seriously considered. Moreover, in the stage just preceding the brief resting phase of the spermatids (Figs. 57, 58 and 59) there occurs a resolution of the five chromosomes into nine and of the four into eight. This demonstrates that the true character of the second division is equational. The second numerical reduction involves a less close union apparently than the first, as a comparison of illustrations 29 and 43 will show. Again the fusion is sometimes incomplete to the extent of giving an occasional count of six chromosomes."

My own belief that the real character of the second division in the case of the pig is simply equational is based on a number of facts. Even after the first maturation division the ordinary chromosomes are much larger than the spermatogonial univalents. This increase in size has evidently taken place during the growth period of the primary spermatocyte (compare Figs. 18 and 38). During the prophase of the secondary spermatocyte the chromosomes apparently increase still more in size and four large bivalent autosomes appear for division in the one type of cell and

four plus the two accessories in the other type. Not only is the bivalent nature of these large autosomes conspicuous, but very frequently a quadrivalent character is discernible and they are much larger than the accessories (Figs. 44, 45, 46 and 47). During the anaphase the bivalent nature of the chromosomes is often clearly visible at each pole. Figure 49 shows four bivalents and is a drawing of one pole of the dividing cell which received eight chromosomes during the first maturation division. Figure 52 is a drawing of one pole of the division of the type of secondary spermatocyte which received eight ordinary chromosomes and the double accessory. It will be seen that four of these are bivalent in nature, while two are univalent, the two univalents being the results of the division of the two accessories (Figs. 44, 50 and 51) both of which have here divided for the first time since the spermatogonial division. The bivalent nature of the autosomes after the second spermatocytic division becomes even more conspicuous in cases where these chromosomes divide into two before they break up. Figure 56 shows an early spermatid cell which received the four bivalent autosomes and the two accessories. It can be seen that two of the autosomes have almost completely divided. The bivalent nature of the other two can also be plainly seen, while the accessories retain their univalent appearance. Thus it can be seen that the spermatids produced by this second division do not receive four and six chromosomes respectively, but, four bivalents, or the equivalent of eight univalents, are present in the one type of cell, and four bivalents or eight univalents together with the two accessories in the other. The foregoing facts seem to indicate beyond doubt that the second maturation division is not a reduction but simply an equational one.

Figures 50 and 54 represent an anaphase of division in a secondary spermatocyte showing two streaks of lagging chromatic material. Although several such cases were observed ordinarily no such pronounced streaks occur (Fig. 51). Guyer found a similar condition in the secondary spermatocyte division in man and suggests that it may be the two accessories lagging behind. Although many accurate counts of the chromosomes in this second division were made, they were so massed together

as to render a count impossible (Fig. 54). Not infrequently the chromosomes in the early spermatids become closely appressed before they begin to break up (Figs. 53 and 57). Sometimes the individuality of the chromosomes is apparent even after the nuclear wall begins to form (Fig. 58).

Spermatids.

From the foregoing evidence it is obvious that there exists a dimorphism among the spermatids, one type containing eight and the other ten chromosomes after the last division. The chromosomes soon become irregular in shape and begin to break up, and the spermatids appear all alike except that immediately after the chromosomes disintegrate two nucleoli are visible in approximately half of the cells (Figs. 64 and 65). These retain for a short time the staining capacity characteristic of the nucleoli of the previous stages, but later disappear and the whole nucleus assumes a coarse granular appearance. The cells seemingly remain quiescent in that condition for some time as they are nearly always found in large numbers. The centrosome surrounded by a clear layer is again very conspicuous within the comparatively small centrosphere which lies close to the nucleus (Figs. 64 and 65).

Development of the Spermatozoa.

Following the period of rest the spermatids begin to develop into spermatozoa. There is no perceptible change in the size of any of the parts, such as described by Jordan in the opossum. The first change to be detected is the extrusion of the centrosome, surrounded by a light area, from the small sphere (Fig. 66). It takes a position a short distance from the nucleus and soon begins to divide. The two new centrosomes move apart and one, somewhat rod-shaped, comes in contact with the nuclear wall but it remains connected by a mass of material with the other centrosome which assumes a disk shape and for a while remains in place (Figs. 67, 68 and 69). The disk is frequently perforated in the middle and has the appearance of a ring (Fig. 70).

Simultaneously with the division of the centrosome, a portion of the sphere which remains in close contact with the nuclear wall

migrates to a point directly opposite the centrosome in contact with the wall on the other side (Figs. 66, 67 and 68). The sphere in this process of migration is in such close contact with the nuclear wall that a slight depression can be detected in the latter. The depression is even more pronounced when the clear sphere becomes definitely fixed as the small acrosome (Figs. 68–75).

In the guinea-pig the acrosome, which becomes nearly as large as the nucleus itself, according to Meves ('99), is also formed from the centrosphere or idiozome. While in the rat, according to Lenhossek, it is independently formed in the cytoplasm without relation to the preceding mitotic figure or the centrosomes. Meves ('97) also found that in the salamander the acrosome is formed from the idiozome which wanders around the nucleus to its anterior pole. McGregor's results on *Amphiuma* ('99) agree in general with those of Meves, except that here the acrosome arises from only a part of the centrosphere, while a second smaller part passes to the base of the nucleus and forms the main part of the middle-piece. Coincidentally with the division of the centrosome and the migration of the small sphere the nucleus together with these structures moves to one side of the cell in the direction of the acrosome and soon practically all of the cytoplasm is at the posterior end of the cell (Figs. 66, 67, 68, 69 and 70). The cell wall seems to persist as a thin mantle covering the head of the spermatozoan.

The anterior part of the cell, bearing the acrosome, almost invariably points in the direction of the tubule wall while the mass of cytoplasm extends into the lumen (Fig. 2). Most of the chromatic material of the nucleus gathers at the center into a dense mass which has the same staining capacity as characterizes the chromosomes and the centrosome. Sometimes two or three masses are present (Figs. 73, 74 and 75).

Soon after the division of the centrosome into a cylindrical anterior and a disc-shaped posterior body, a divergence of the two follows, but they remain connected by a streak of material (Fig. 69). The inner body upon coming in contact with the nuclear wall forms a depression in the latter and its anterior portion shapes into a disc which comes in close contact with the nuclear wall in the small depression at the posterior end of the

nucleus. From this disc, which is apparently the end-knob, a cylindrical mass of material extends backwards uniting the two centrosomes (Figs. 68 and 69). A tiny filament extending backward from the middle of the posterior disc-shaped centrosome makes its appearance and is undoubtedly a continuation of the coarser filament which unites the two centrosomes (Fig. 69). As the tail grows longer the connecting filament becomes thinner, and the posterior centrosome, which after division was disc-shaped and had increased somewhat in size, becomes transformed into a ring and it can be seen that the filament extends directly through it and continues backward as the tail (Fig. 70). The formation of the ring takes place simultaneously with the rapid growth of the tail and one is led to believe that the perforation in the disc is partly due to the fact that the material formerly occupying that space goes to help in building up the axial filament. Shortly after the tail projects out of the cell the ring moves along the filament, backward, and soon swerves over to one side in the cytoplasmic mass (Figs. 71 and 72). Very frequently it can be seen a considerable distance away from the axial filament long before the latter is fully developed (Figs. 73, 74 and 75). This seems to indicate that the posterior centrosome takes no further part in the development of the filament and that the latter is mainly developed from the anterior centrosome, no part of which is discarded or thrown off. While most of the inner centrosome passes into the formation of the axial filament a part of it remains as the end-knob in the small middle-piece (Figs. 76 and 78), a condition similar to that found by McGregor in *Amphiuma* ('99).

The posterior ring-shaped centrosome, after moving away from the filament, sometimes divides (Fig. 73), but usually assumes a spherical shape (Fig. 74) and an interesting point in connection with this body is that during the final development of the spermatozoan it is invariably thrown off with a big mass of cytoplasm (Figs. 74, 75 and 76). The casting off of a portion of the cytoplasm during the last stages of the developing spermatozoan has been described by Meves ('99) in the guinea-pig where it is closely similar to the process which occurs in the spermatozoid-formation in ferns; but the throwing off of a portion of the

centrosome together with the mass of cytoplasm has not heretofore been, to my knowledge, recorded. The body usually stains as deeply as it does in the earlier stages for some time after the comparatively large mass of cytoplasm containing it is completely separated from the rest of the cell or young spermatozoan.

In general, the behavior of the centrosomes in the development of the spermatozoan of the pig does not differ greatly from the conditions found in some of the other vertebrates. However, it might be well to briefly point out some of the differences. In the spermatids of the salamander, according to Meves ('97), the two centrosomes lie quite at the periphery of the cell and from the outer one grows out the axial filament. The two centrosomes leaving the idiozome by which they are first surrounded, now pass inwards toward the nucleus, the outer one meanwhile becoming transformed into a ring while the axial filament passes through it to become attached to the inner centrosome. The latter pushes into the base of the nucleus and enlarges enormously to form a cylindrical body comprising the main portion of the middle-piece. The ring divides into two parts, the anterior of which gives rise to a small body at the posterior end of the middle-piece identical with the end-knob. The other part of the ring wanders out along the tail and finally lies at the limit between the main part of the latter and the end-piece.

In the pigeon, according to Guyer ('00), the centrosome divides and moves out of the sphere and further away from the nucleus. The two new centrosomes move apart, but remain connected by a mass of material which later disappears and a very delicate fibril uniting the two centrosomes exists in its place. One of the centrosomes enlarges and transforms into a complete ring. The connecting fibril can later be seen passing from the smaller centrosome back through the ring and outside the cell. The centrosomes next approach the nucleus and as they draw near a slight invagination appears in the nuclear wall and the small centrosome moves into it.

In the mammals, the work of Lenhossek on the rat ('98) and Meves on the rat, guinea-pig and man ('98, '99) gives a result similar to the condition found in the salamander. In all these mammals the young spermatids contain two peripherally placed

centrosomes, from the outer one of which the axial filament grows out and the centrosomes later move toward the nucleus. In the pig the centrosomes are never peripherally located and division of the spermatid centrosome does not take place until it emerges, surrounded by a small clear sphere, from the main bulk of the centrosphere which is in close contact with the nucleus.

It can be seen from the drawings of the later stages that the nucleus gradually elongates and flattens (Figs. 67–78). The large chromatic mass within the nucleus disintegrates and the particles become distributed at the periphery of the nuclear wall (Fig. 78). The tail envelope becomes apparent as the cell elongates and evidently develops from the cytoplasm (Figs. 74–78). It later comes in contact with the axial filament and envelops about half of its entire length. The remaining extremely thin portion of the tail is apparently the naked axial filament (Figs. 76, 77, 78).

When the cells reach the stage represented in Figs. 71–73 they attach themselves in bunches to the large cylindrical Sertoli or nurse cells that often extend from the basement membrane a long distance toward the lumen of the tubule (Figs. 2 and 63). As the spermatozoa continue to develop a gradual decrease in the volume of the cytoplasmic mass of the Sertoli cells is noticeable. When the spermatozoa are apparently mature they abandon the Sertoli cells which at this time are very much collapsed. The spermatozoa after leaving the nurse cells do not pass directly to the lumen of the tubule, but remain scattered for some time among the masses of cytoplasm which were cast off by the same developing cells a short time before (Figs. 3 and 67). This cast-off material does not scatter about in the lumen, as one would suspect, but forms a sort of loose layer next to the cells following the maturing spermatozoa. The masses begin to disintegrate soon after they are discarded by the developing spermatozoan, and when stained with iron-hæmatoxylin numerous black bodies make their appearance in this material (Fig. 3). The spermatozoa remain, with their bodies embedded in this layer, and tails extending into the lumen, until the mass almost completely disappears. Then they become free in lumen of the tubule and are ready to make their way out of the testes. During this period

of attachment to the nurse cells and later to the discarded mass of cytoplasm the heads of the spermatozoa increase somewhat in size but retain the same staining capacity. The foregoing facts seem to show beyond doubt that the developing spermatozoan derives nourishment not only from the nurse cells but also from the cast-off cytoplasmic material. When the sperm reaches maturity the acrosome disappears from view and the anterior edge of the head becomes well rounded.

In addition to the parts mentioned, there appears very frequently a dense spherical cytoplasmic mass represented in Figs. 67 to 75. As to the origin and fate of this body I am not entirely certain. It seems, however, that it is a portion of the centrosphere, for in many of the early stages it was seen in the immediate neighborhood of the small body which later forms the acrosome (Fig. 67). Further evidence for this assumption is the fact that the combined mass of these two bodies seems to be equal to the size of the sphere in the stage immediately preceding (Fig. 66). The consistency of this body, too, is similar to that of the sphere.

Figure 78 represents a mature spermatozoan of the pig which appears rather simple in structure and form. The entire nucleus of the spermatid has evidently developed into the head, which is oblong and flat (Figs. 70–78). The nuclear material breaks up into very fine particles which arrange themselves in a layer at the entire periphery of the head wall (Fig. 77).

With Heidenhain's iron-hæmatoxylin the head stains a sort of slate blue and is difficult to decolorize. A depression is present at the posterior extremity which is in contact with a small middle piece. The end-knob which is for some time attached to the nuclear wall within the small depression (Figs. 74, 75 and 76) finally breaks loose and passes to the posterior extremity of the middle piece, the greatest portion of which now remains very clear in appearance. This clear area is similar in appearance to the contents of the acrosome and one is led to believe that it is due to the presence of the substance which was seen in form of a clear sphere surrounding the centrosome after it emerged from the centrosphere and persisted about the two centrosomes, particularly around the inner one, after division (Figs. 66–69)

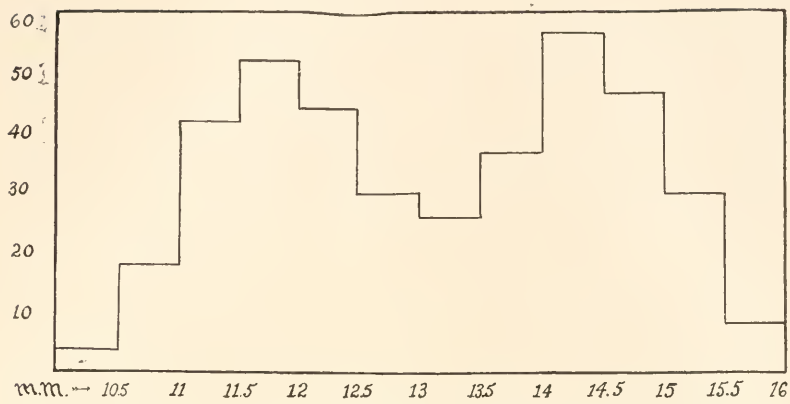

FIG. 1. Diagram showing the variation in size among four hundred mature pig spermatozoa. Figures at the left give the numbers of the individuals belonging to each type. Figures at the bottom give the lengths of the heads of the spermatozoa in millimeters, magnified two thousand times.

VARIATION IN SIZE OF MATURE SPERMATOZOA.

The spermatozoa of the pig vary considerably in size, and careful measurements revealed the fact that they may be arranged in two separate classes, one type being much larger than the other. Mature specimens, which were free in the lumen of the tubule and parallel to the objective, were selected at random, and outline sketches of four hundred heads enlarged (× 2,000) were made with the aid of a camera-lucida. The lengths of the sketches were then carefully measured and recorded in half millimeters. Figure 1 in the text shows the variation in size of the four hundred heads measured. It can be seen at a glance that two separate types of spermatozoa exist; the greatest number of the one kind measuring from 11.5 to 12 mm., and of the other type from 14 to 14.5 mm. The increased size of the latter is due presumably to the presence of the accessory chromosomes.

RELATION OF THE ACCESSORY CHROMOSOMES TO SEX IN THE PIG.

Many investigators working on the problem of sex determination are of the opinion that the sex of a zygote is determined at the time of fertilization, the spermatozoan in most cases carrying

the determining factor. This view has been recently substantiated by the careful investigations of McClung, Stevens, Wilson, Morgan, Payne and others, who found that in many invertebrates such as certain insects, myriopods, arachnids and nematodes, two different kinds of spermatozoa are produced. The spermatozoa differ from each other by one chromosome, or by a group of chromosomes, called the accessory chromosomes, or X-chromosomes.

In some forms it was found that the spermatozoa each contain an accessory chromosome, but that in half the sperms this chromosome was larger in its chromatin content than in the other. These are called the X and Y elements respectively. All the eggs produced (except in a few cases), on the other hand, contain the X-element.

It has been shown in some of the Tracheata that whenever an egg is fertilized by a sperm containing the X-chromosome it develops into a female, while an egg fertilized by a sperm without the X-element, or by one containing the Y-element, gives rise to a male individual.

In the vertebrates, Guyer was able to identify an X-element in such diverse forms as the guinea, rooster, pigeon, rat, and man, and other investigators have recently brought forth similar evidence. Patterson and Newman ('10) record its presence in the armadillo, Jordan ('11) in the opossum, Stevens ('11) in the guinea-pig, and King ('12) in *Necturus*. Up to the present investigation, however, a dimorphism in the number of the chromosomes in the male and female vertebrates has not been recorded. Guyer ('10) after speaking of the difference in the number of chromosomes in the somatic cells of the male and female Tracheata which possess the X-elements says:

"In the light of these facts we should expect the somatic cells of man to contain twenty-two and of woman, twenty-four chromosomes. The tissues of the female have not yet been studied with this in mind. Flemming ('97) records the somatic number of chromosomes, determined from corneal cells, as twenty-four but unfortunately he does not record the sex of the subjects from which the material was obtained. If it were a female his count would bear out the interpretation given above."

The behavior of the accessory chromosomes in the various stages of the spermatogenesis of the pig further substantiates the fact that two different kind of spermatozoa are produced in mammals. Since the dimorphic condition of the spermatozoa does exist, the question arose whether this dimorphism holds true in the chromosome number of the male and female somatic cells. With this in view I undertook an examination of sections of male and female embryological material supplied me by Professor B. M. Allen. A large number of these sections were studied and although mitotic stages were not very abundant in any one section, and the chromosomes were frequently bunched together rendering a count impossible, nevertheless, a number of counts of the chromosomes in the germinal and somatic cells of the two sexes were recorded. It was again found that the spermatogonial number of chromosomes is eighteen, and that the same number prevails in the somatic cells found in the mesonephros of the male embryos. Two of the chromosomes are usually somewhat larger (Fig. 59). Sections of the female material revealed a count of twenty chromosomes in the oögonia (Fig. 60), and the same number prevails in the somatic cells found in the mesonephros of the female embryos (Fig. 62). Four large chromosomes corresponding to the two accessories in the male can usually be detected in the oögonial cells. In the somatic cells of the female there are also four large chromosomes present corresponding to the two accessories in the male cells and the four accessories in the oögonia (Fig. 62).

In a few cases ten large chromosomes were found in the early metaphases of division in the somatic cells of the female (Fig. 61). Two of these are considerably larger and are interpreted as the result of the pairing of the four accessories. The smaller chromosomes, eight in number, judging by their size, are evidently due to the pairing of the sixteen other chromosomes. The position of these cells would not warrant the supposition that they might be wandering germ cells. Furthermore, a similar condition was not observed among the oögonial cells in spite of the fact that mitotic stages occur far more frequently in the sections of the ovaries than they do in the mesonephros.

In proportion to the large number of mitotic stages found in

these long-continued searches, accurate counts of chromosomes in the cells of the various tissues were comparatively few. However, all cases where a count was possible were recorded and although they varied somewhat, the above number of chromosomes, eighteen and twenty, attributed to the male and female cells respectively, were the prevailing numbers.

The foregoing facts are in accord with the expectations, and we have here in a vertebrate a condition verifying the results found in some of the lower forms. In view of these facts it is obvious that the eggs, carrying ten chromosomes, or half the somatic number, when fertilized by a spermatozoan containing ten chromosomes, give rise to an individual containing twenty chromosomes in its cells, or a female. Those fertilized by the other type of spermatozoan, which contains only eight chromosomes, give rise to individuals with eighteen chromosomes in their cells, which was found to be true in the male.

The results of the present investigation, therefore, add support to the chromosome theory of sex determination, since they show that in the vertebrates, as well as in some of the lower forms, there exists a dimorphism in the number of chromosomes in the somatic as well as the germinal cells of the two sexes. It is highly probable that conditions similar to those found in the pig, as regards sex determination, exist in man and in the other vertebrates which possess the accessory or X-chromosomes. The resemblance in the behavior of the pair of accessories in the pig and their behavior in man is very striking and suggests that in all probabilities there exists a dimorphism in the germinal and somatic cells of man and woman.

Summary.

1. The usual four types of cells, the spermatogonia, primary and secondary spermatocytes, and spermatids are discernible in the spermatogenesis of the pig.

2. Large interstitial cells containing numerous mitochondria exist in great abundance and comprise about one fourth of the entire mass of the testes.

3. Numerous Sertoli or nurse cells are present and great numbers of spermatozoa can be seen in all stages of development in the various tubules.

4. Two large nucleoli are present in the resting stages of the spermatogonia. These can be traced through the entire spermatogenesis of the pig and are apparently correlated with, or are the same thing as the two accessories. Smaller nucleoli, usually two, are also present.

5. Eighteen rod-shaped chromosomes differing somewhat in size occur in all of the spermatogonia where a definite count could be made. Two of these, undoubtedly the accessories, can usually be seen to one side of the main mass of chromosomes.

6. During the spermatogonial division the accessories divide and occasionally pass to the poles in advance of the other chromosomes.

7. The last spermatogonial division gives rise to cells which through a process of growth become the primary spermatocytes. Both nucleus and cytoplasm increase greatly in size. Synizesis followed by synapsis occurs.

8. An apparently continuous spireme is formed which later breaks up into U-shaped and variously curved chromosomes. A centrosphere containing the centrosome is present.

9. The two large round nucleoli which remain very conspicuous throughout the process of growth of the primary spermatocyte come together and elongate during the late prophase.

10. Ten chromosomes appear for division in the primary spermatocyte of which eight are evidently bivalent and two accessory. The accessories are usually considerably to one side of the other chromosome.

11. The two accessory chromosomes which are out of the main spindle pass undivided to one pole in advance of the other chromosomes.

12. The primary spermatocyte division is evidently the reduction division. It gives rise to two cells, one of which contains ten (eight autosomes plus the two accessories) and the other only eight chromosomes.

13. The chromosomes in these daughter cells are larger than the univalents of the spermatogonia and show some indications of bivalence.

14. The secondary spermatocytes formed by the last division ordinarily go into a resting stage. In half of these the two

nucleoli are conspicuous and lacking in the others. In a few cases, at least, there is no resting period in the secondary spermatocytes. A small centrosphere containing a relatively large centrosome is present.

15. During the metaphase of the secondary spermatocyte one half show four large bivalent chromosomes, and the remaining show the four large bivalents plus the two accessories or six chromosomes. A second pairing has apparently taken place but the division is simply equational as the four large chromosomes often manifest a quadrivalent character. The accessories remain unpaired.

16. The quadrivalent nature of the autosomes in the secondary spermatocytes becomes all the more certain after division, as the chromosomes passing to the poles are of the bivalent nature.

17. The type of secondary spermatocyte which received eight chromosomes after the first maturation division gives rise to two spermatids each containing four bivalent or eight univalent chromosomes. The other type which received ten chromosomes after the first maturation division gives rise to two spermatids, each containing four bivalent or eight univalent chromosomes and the two accessories, each accessory having divided here for the first time since the spermatogonial division.

18. The dimorphic nature of the spermatids which develop into spermatozoa is further evinced by the presence of the two nucleoli in approximately half the cells. The centrosome is again conspicuous.

19. The first noticeable change in the transformation of the spermatid is in the centrosome. It emerges from the small sphere and divides into an anterior rod-shaped and a posterior disc- or ring-shaped body.

20. Both of the centrosomes contribute to the development of the axial filament. A portion of the anterior one persists as the end-knob, while the posterior body is finally cast off.

21. A portion of the sphere migrates to the opposite side and gives rise to the acrosome which disappears when the spermatozoan is fully developed.

22. The nucleus of the spermatid passes to one side of the cell, elongates and flattens and forms the head of the spermatozoan.

23. The axial envelope which extends a little more than half the length of the tail is developed from the cytoplasm.

24. The half developed spermatozoa attach themselves to Sertoli cells where they continue to develop.

25. The cytoplasmic mass of the Sertoli cells decreases greatly in size as the spermatozoa continue to develop. Finally, when the spermatozoa are almost mature, a collapse of the nurse cells takes place when practically nothing but the nucleus and the cell wall remain.

26. An interesting event occurs in the final stages of the development of the spermatozoan when a large mass of cytoplasm together with the posterior centrosome is thrown off by the cell.

27. When the spermatozoa desert the Sertoli cells they do not pass directly into the lumen of the tubule but remain scattered with their heads embedded in the layer of the cast-off cytoplasm.

28. The cast-off cytoplasmic material is apparently used as food by the maturing spermatozoa and when practically all of the former disappears the fully developed spermatozoa become free in the lumen of the tubule.

29. Measurements of the mature spermatozoa reveal the fact that they are of two distinct sizes, a point no doubt correlated with the presence and absence of the accessory chromosomes.

30. The somatic cells of the male contain eighteen chromosomes, a number corresponding to that in the spermatogonia.

31. The somatic cells of the female yield a count of twenty chromosomes as do the oögonia.

32. The foregoing facts add considerably to the support of the chromosome theory of sex determination, since they prove that in the vertebrates, as is true of some invertebrates, there exists a dimorphism in the germinal and somatic cells of the male and female.

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EXPLANATION OF PLATES.

Plate I.

(Fig. 1, ×200; Figs. 2 and 3, ×500; Figs. 4–12, ×1,200.)

1. Section of a pig testis showing the seminiferous tubules separated by wide areas of interstitial cells.

2. Section of a tubule showing bunches of developing spermatozoa attached to Sertoli or nurse cells.

3. Section of a tubule showing almost fully developed spermatozoa which have deserted the Sertoli cells and became scattered among the masses of cast-off cytoplasm.

4. Resting stage of a spermatogonial cell showing two large nucleoli.

5. Primary spermatocyte in the synizesis stage.

6. Resting stage of a primary spermatocyte showing the two large nucleoli.

7. Spireme stage of a primary spermatocyte showing the chromatin threads and the two persisting nucleoli.

8. Late prophase of division in a primary spermatocyte showing eight chromosomes in the main group, and the two accessories lying off to one side. The photograph does not reveal the full size of the accessories.

9 and 10. Side views of primary spermatocytes showing the cell ready for division with the accessories lying just above the level of the regular equatorial plate of chromosomes.

11. Side view of a primary spermatocyte division with the two accessory chromosomes passing to the pole, side by side, in advance of the other chromosomes.

12. Side view of a primary spermatocyte division showing the two accessories at the pole while the autosomes are still in the equatorial plate undivided.

13. Two metaphases of secondary spermatocytes showing four chromosomes in the equatorial plate. The figure at the top is a side view, and the one at the bottom a polar view.

14. The cell at the top is one type of secondary spermatocyte which contains four chromosomes in the metaphase stage and the cell below is a spermatid resulting from the division of a type of secondary spermatocyte which received eight autosomes plus the two accessories.

15. Resting stage of a spermatid showing two nucleoli.

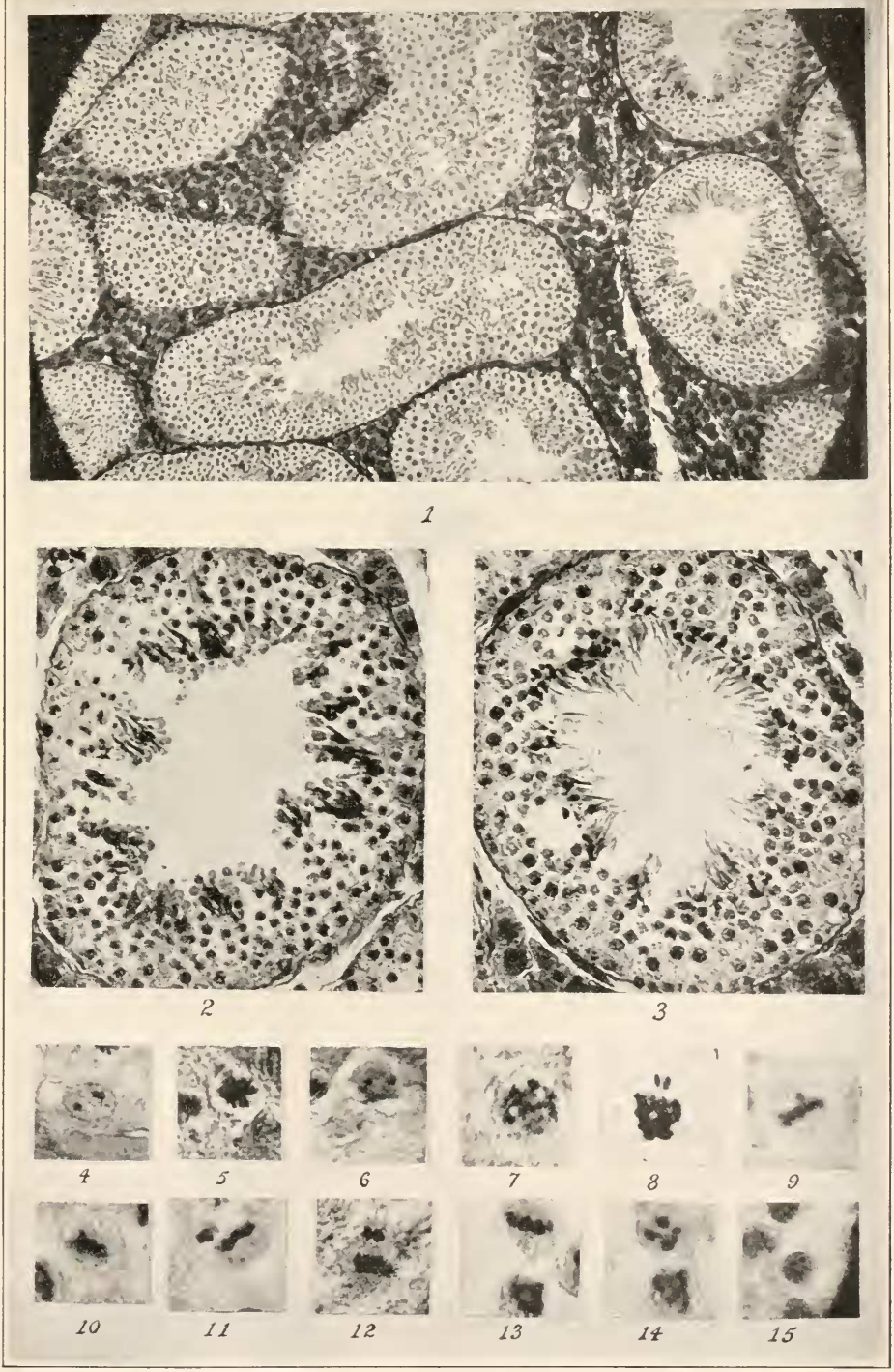
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Plate II.

(All of the drawings on plates 2–6 were made with the aid of a camera lucida. ×2,330.)

16. Early spermatogonial cell showing two large nucleoli. The cell boundary is quite indistinct.

17. Resting stage of a full grown spermatogonial cell showing two large and two small nucleoli.

18. Late prophase of a spermatogonial division showing eighteen chromosomes. The two large oval chromosomes at the top are apparently the accessories.

19. Metaphase of division in a spermatogonium showing the two divided accessories at the right.

20. Early resting stage of a primary spermatocyte showing the centrosome, two large, and several small nucleoli.

21. Primary spermatocyte in synizesis.

22. Primary spermatocyte following synizesis and synapsis; the entangled mass of threads moved toward the center of the nucleus and the threads appear to be twice as thick as those in the synizesis stage.

23. Primary spermatocyte following the synaptic stage. The clear area in the nucleus disappears and the nuclear wall becomes well developed.

24. Spireme stage of a primary spermatocyte showing increase in size of both the nucleus and the cytoplasm and also the two large nucleoli.

25. Breaking up of the spireme into U and variously shaped chromosomes. The two nucleoli become oval in shape and can always be seen close together in this stage.

26, 27 and 28. Late prophases of primary spermatocytes showing ten chromosomes. The two oval accessories, which are smaller than the bivalent autosomes, are shown in characteristic positions.

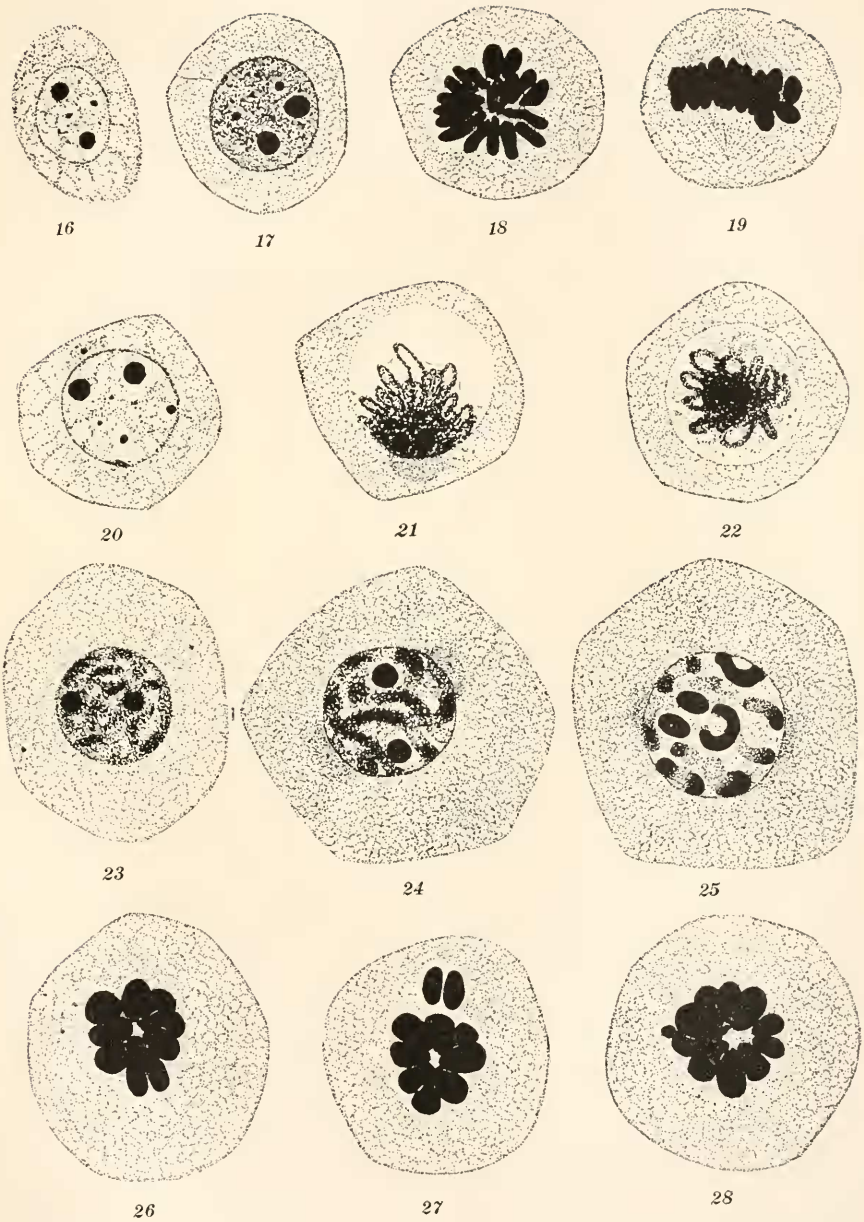

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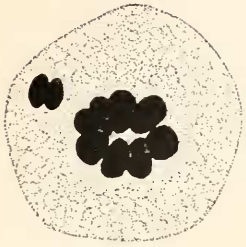
PLATE III.

29. Late prophase of a primary spermatocyte showing ten chromosomes, the two accessories lying a considerable distance away from the regular equatorial plate.

30–37. Metaphase of division in primary spermatocytes showing the two accessory chromosomes in characteristic positions passing to the poles. Figures 31 and 32 show also a small body passing to the same pole with the accessories, Figure 35 shows it passing to the opposite pole; and Fig. 37 shows two such bodies, one passing to each pole, which is a case of extremely rare occurrence.



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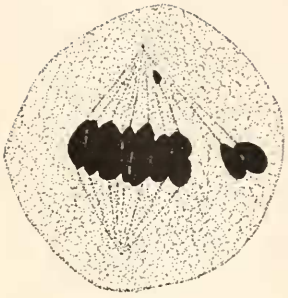

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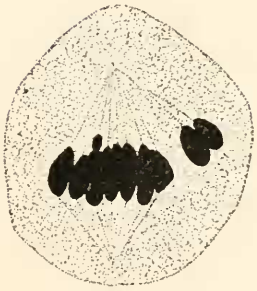

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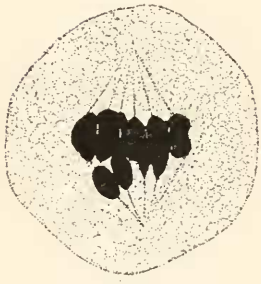

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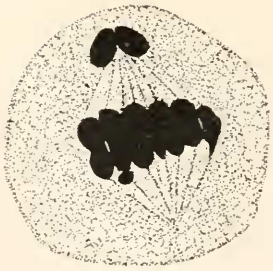

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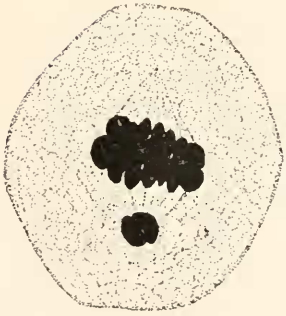

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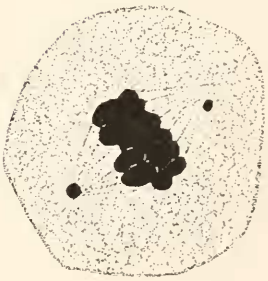

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